THE OCCURRENCE AND NATURE OF POLYPLOIDY IN A COLCHICINE TREATED POPULATION OF SUGAR BEETS

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ABSTRACT

Chromosome counts were obtained from heart leaves (2nd, 4th, 12th, 13th, 19th and 20th) of colchicine treated sugar beets plants and correlated with the chromosome counts of pollen mother cells with a view to establishing a method of selecting only 4N inflorescences. It was found that counts of the 12th or a subsequent heart leaf in conjunction with counts of a floral heart leaf were satisfactory indicators of the ploidy level of the inflorescence. The number of chloroplasts per 2 guard cells was not a satisfactory index for selection of 4N inflorescences due to the presence of periclinal chimeras. Pollen diameter was found to be a workable selection criterion for the determination of the ploidy level of the inflorescence.

Root tips were relatively less affected by colchicine than the shoot apex and gave no indication as to the type of inflorescence the plant would produce.

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INTRODUCTION

The yield advantage of triploid sugar beets over diploids (30) and the possibility of using male sterility to facilitate the production of hybrid triploids has increased the need for the efficient production of large numbers of tetraploid strains.

Although an effective method for polyploid induction is established, controversy exists as to the best means of identifying tetraploid plants in the Co (treated) generation. Fortunately the type of growth of the sugar beet lends itself to such an investigation. The leaves of the shoot apex grow very vigorously and chromosome counts can easily be obtained on leaves up to 15 mm. in length. Also it is possible to determine the chromosome number of the Co generation by investigation of pollen mother cells (PMC's). Thus one can follow the effect of colchicine on the chromosome number of prefloral growth via cytology of the heart leaves and correlate this with the chromosome number of the C_1 generation.

The "tunica corpus" concept of apical meristem organization visualizes the promeristem to consist of two independent layers (24). Evidence of periclinal chimeras in dicotyledons supports this hypothesis (6). The outer tunica consists of two layers which give rise to the epidermis by anticlinal divisions. The corpus which is enclosed by the peripheral tunica gives rise to the vascular and ground tissue by periclinal and anticlinal divisions. There may be, however, some modifications of this general scheme as the layers of the tunica and corpus sometimes give rise to tissue other than that designated above (7).

Theoretically colchicine could have a range in effectiveness of polyploid induction from complete conversion to no effect. However, even if all of the initials are not converted to the 4N condition, a certain percent conversion may result in a 4N inflorescence if the 4N initials in such a chimera give rise to the floral apex.

Since both the heart leaves and the inflorescence are believed to arise from the corpus, the chromosome "balance" in one should be correlated with the other. One can assume that the leaves are a random sample of the shoot apex since the heart leaves arise from the promeristem in accordance with the phyllotaxis of the shoot (2/5).

The presence of mixoploid or sectorial chimeras could lead to confusing results as the primordia that initiate the heart leaves could come from a different area of the corpus than that which initiates the flowers. Periclinal and mericlinal chimeras could invalidate the use of chloroplast counts of epidermal guard cells as an indicator of chromosome number in the germ plasm. A difference in the rate of division of 2N and 4N cells in the corpus initials would cause the chromosome count at one growth stage to be different than at a subsequent growth stage. Similarly a difference in the rate of division of 2N and 4N cells in the young heart leaf could give a false indication of the chromosome number of the corpus. Another probable source of experimental error is from an inadequate sampling of the heart leaves and inflorescences.

With the above statements in mind a study of the effect of colchicine on the chromosome number of plant growth was made with the object of determining selection criteria that would permit accurate identification of plants in the vegetative state which would produce only tetraploid seed when induced to flower.

LITERATURE REVIEW

Several methods, other than the counting of chromosomes, have been used to predict the ploidy level of sugar beet plants. Due to the frequent occurrence of chimeras in the Co the selection criteria that are suitable in later generations may not be useful in the Co (29).

Artschwager (2) used plant morphology in the Co in conjunction with other selection criteria to evaluate the efficiency of tetraploid induction. Savitsky (29), however, found plant morphology alone to be a poor criterion. Of the plants that appeared to be affected by colchicine in the seedling stage only 6.4 percent produced 4N inflorescences.

Stomata size was used by different workers (1, 2, 3) as a screening devise, but due to the applied nature of their investigations they did not give experimental evidence to show it was a valid criterion. Peto and Hill (21) suggested using the area index of the stomata from comparable leaves as a selection criterion but gave no data to substantiate its value. Deneuche (5), Varga (32) and others (10, 26) indicated that stomatal size is a poor selection criterion.

Evidence has been given (4, 9, 20) that the number of chloroplasts per guard cell is positively correlated with the ploidy level of the plant. Powers and Dudley (22) suggested this as a method for screening Co seedlings in order to eliminate undesirable plants. Savitsky (29), however, found that of 225 Co plants whose inflorescences were shown to be 2N via pollen diameter, 125 (55.5%) had a larger number of chloroplasts than is expected in diploids. Similarily of 361 Co plants whose

inflorescences were shown to be 4N via pollen diameter, 86 (23.6%) had a lower number than is expected in 4N guard cells. Kloen and Speckman (17) and Rosen (26) emphasized that there is a variable environmental effect on the number of chloroplasts per guard cell.

Many workers (1, 2, 11, 12, 27, 31) have indicated that the increased diameter of 4N pollen grains could be used to select for inflorescences with diploid gametes but several workers (10, 17, 26, 32) have found this method unsatisfactory. Kuzdowicz (18) and Walther (33) state that the number of pores per pollen grain can be used to differentiate between haploid and diploid pollen grains. Walther (33) claims that environment produces a smaller variability in pore number than it does on pollen diameter. However, Varga (32) found this method unsatisfactory and Essad and Touvin (10) and Dona'Dalle Rose (8) found it less satisfactory than chloroplast or chromosome counts.

The number of nucleoli in the resting nucleus of epidermal cells has been used by Reitberger (25) and Graf (14) to differentiate between 2N, 3N and 4N plants. Speckman (31) found that the time saved in analysis by this method was not great enough to compensate for the extra time it took to make the preparations. Similarily Varga (32), Essad and Touvin (10) and Dona' Dalle Rose (8) found this method unsatisfactory.

Feltz (11) suggested that higher amounts of abnormal tetrad formation in 4N plants could be used as a selection criterion. However, he stated that the greater amount of non-staining micro pollen grains found among the diploid pollen grains could not be used as an absolute

selection criterion.

Kloen and Speckman (16, 17) found that cytological examination of leaves in the rosette stages was an ineffective method of selecting for 4N inflorescences in the Co. They then examined the cytology of heart leaves of the floral apex from plants previously selected for 4N tissue in the prebolting heart leaves and excised the apices that were not 4N at both sampling times. The progeny (C_1) from plants sampled twice gave 77.3 percent 4N, 20.6 percent 3N and 2.1 percent 2N whereas with only one prebolting cytological examination they got 52.6 percent 4N, 45.6 percent 3N and 1.8 percent 2N. They used a rapid counting method in which there is no staining and the material is observed by aid of phase microscopy. Deneuche (5) using the same method, compared its accuracy in estimating chromosome number with the chloroplast number per guard cell. He made 36 errors out of 142 plants examined using the rapid counting method whereas he made only 3 mistakes using the criteria of chloroplast number per guard cell.

Hammond (15) selected 4N plants based on cytology of the floral apex, that were shown to be highly 4N chimera on the basis of vegetative tissue, and found seed from selected plants to be all 4N.

MATERIALS AND METHODS

The material used in this experiment was an open pollinated monogerm strain of sugar beets designated as 6210, obtained from the Sugar Beet Breeding Station, Taber, Alberta. Five hundred seeds were treated with the fungicide, Arasan, and germinated. When 10 percent of the seeds showed protruding root tips the entire sample was placed in a solution of .3 percent colchicine for 6 hours at room temperature, then washed twice in water and planted in the greenhouse.

As the seedlings grew an attempt was made to sample each of the 2nd, 4th, 12th, 13th, 19th and 20th heart leaves (Plate I. Fig. 1-6). However, in the early stages of growth the deliterious effect of colchicine on plant growth made it impossible to always sample the exact heart leaf desired. Thus the first two growth stages were in some instances sampled at the 3rd, and 5th or 6th heart leaf. Since the prime interest of the experiment was not to determine an effective method of polyploid induction but rather to determine the nature of colchicine conversion, seedlings that did not show the morphological effects of colchicine were not sampled and were discarded.

The heart leaves were excised when they attained a length of four to six millimeters. Great care was taken in excising the heart leaves to avoid mechanical damage to the plant apex which would result in abnormal plant growth and thus would not give a true indication of the effect of colchicine on normal plants. However, many plants still had to be discarded because of apical damage which resulted in plants with several apices.

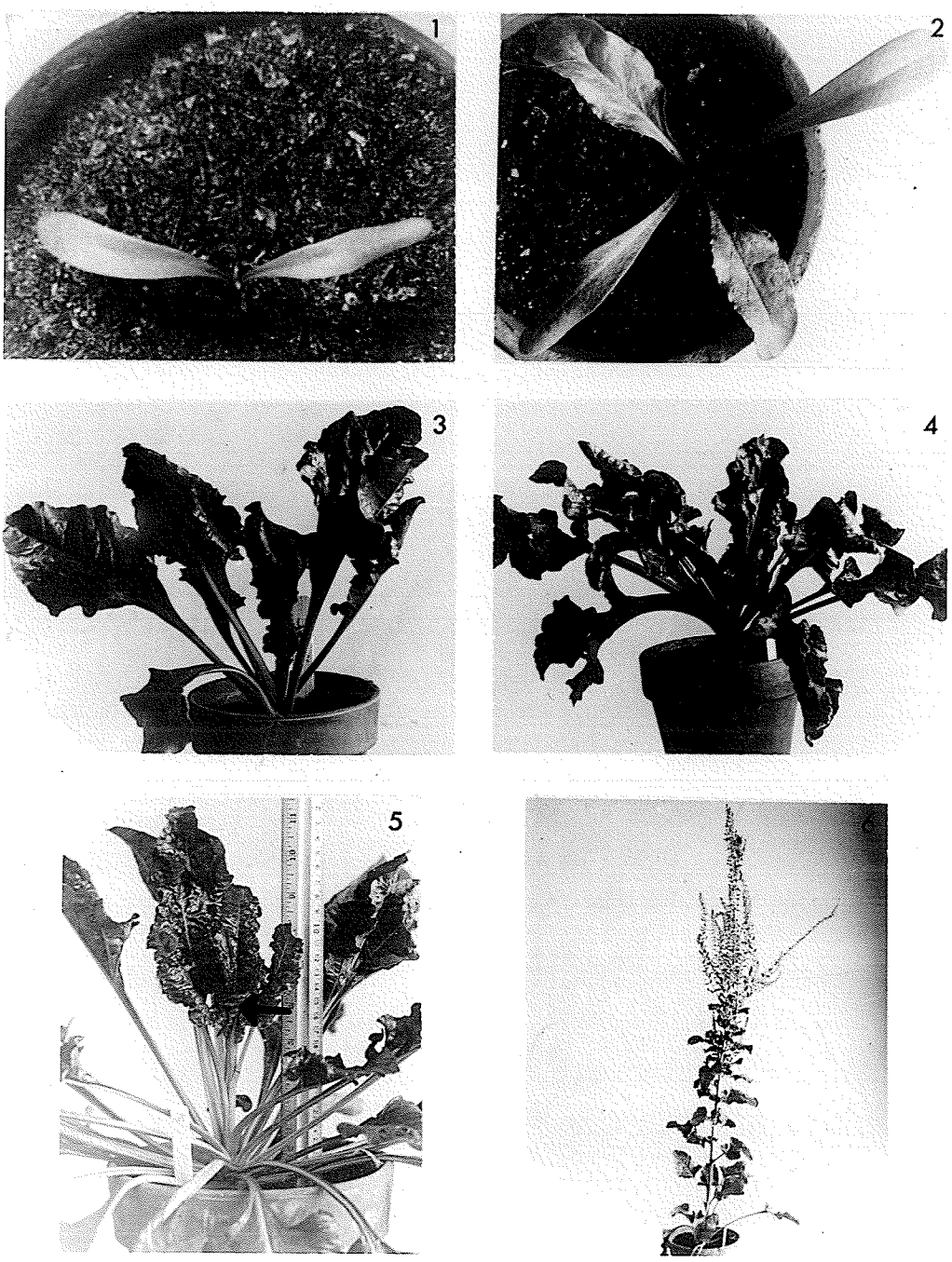

PLATE I - The growth stages from which samples for cytological analysis were taken.

Fig. 1 - two leaf stage

Fig. 2. - four leaf stage

Fig. 3. - 12th leaf stage

Fig. 4. - 20th leaf stage

Fig. 5. - floral apex leaf stage

Fig. 6. - inflorescence - stage from which pollen mother cells were taken.

From each heart leaf collected two random samples were taken from opposite sides of the leaf. This was facilitated by the fact that the periphery of the heart leaf was found to be the area of most active division. Chromosome counts of twenty five cells were made on each sample to give a total of fifty counts per heart leaf.

The heart leaves were collected directly into cold water and received a 24 hour pretreatment at 0° - 2°C. They were then fixed in Farmer's solution (3 parts C_2H_5OH :1 part CH_3COOH) for a minimum of two days. They then received an 8 minute hydrolysis in 1 percent HCl at 60°C. prior to staining in Feulgen. Squash preparations were made using aceto-carmine as the counter-stain.

On the basis of the cytology of the last heart leaf sampled, the plants were divided into 3 groups: diploids, tetraploids and chimeras. These plants then received a photo-thermal induction period of continuous light at 40°F. for three months (13). After the induction period one heart leaf was taken from the floral apex of each plant when the apex was approximately six inches high. Fifty cells per floral heart leaf were counted.

Pollen mother cell counts were made on all plants that bolted. Two random samples were taken from each inflorescence and fixed directly into Carnoy's solution (6 parts C_2H_5OH: 3 parts $CHCl_3$: 1 part CH_3COOH). When a count was made all five stamens were included in the squash preparation. Twenty-five counts were made per slide for a total of fifty counts per inflorescence.

A sample of pollen from one flower of each plant was collected into a solution of 1 part 1 percent aceto-carmine and 1 part glycerol and the diameter of ten viable pollen grains was recorded. The viable pollen grains were defined as those that stained red whereas the inviable remained colorless. It was deemed necessary to do this because the high proportion of aneuploidy in an autotetraploid results in an appreciable amount of inviable pollen grains, of a smaller diameter (11). In addition to this, 2 additional samples of pollen were taken from 2 random flowers from ten plants of the 4N and chimera groups.

Thus a total of three ploidy determinations were taken on all of the inflorescences (2 via PMC's and 1 via pollen diameter), and five determinations on ten plants from each of the 4N and chimera group (2 additional via pollen diameter).

An attempt was also made to count one root tip of each plant that received the photo-thermal induction period. In addition to this a separate population of 106 seeds of strain 6210 were treated with colchicine and root tips were collected from the seedlings. Chromosome counts were obtained by the same technique as described for heart leaves.

The lower epidermis of two leaves (3 inches in length) from ten plants of each of the three groups was stripped into a 1 percent solution of AgCl. The total number of plastids contained in the two guard cells surrounding each of 10 stoma was recorded. Thus there were 200 chloroplast counts per group.

In the case of chimeras an attempt was made to sample prebolting heart leaves at two stages of growth. This was accomplished by excising the leaf tip when the leaf was 4 - 6 mm. long and then excising the remainder of the leaf four days later.

RESULTS AND DISCUSSION

Sixty one percent or 305 of the 500 treated seeds germinated; of these 64 died as seedlings prior to sampling, 118 were discarded because of apical damage during sampling and 51 were discarded because they showed no morphological effect of colchicine. Chromosome counts from heart leaves of plants that later showed apical damage are included in some of the data. The remaining 72 plants were given a photo-thermal induction treatment after being sampled for cytological analysis.

In the course of the cytological analysis difficulty was encountered in obtaining suitable preparations at the early growth stages probably as a result of the deliterious physiological effect of colchicine on early plant growth. Consequently only 10 plants were analysed at all six growth stages; 22 plants at 5 growth stages, nine at four stages and the remaining 31 plants were examined at three or less stages.

Cytological data for the first three groups is presented in Figures 7 to 9 respectively. In Figures 7A, 8A and 9A the average chromosome number per cell at each stage of analysis is recorded; firstly for the entire group and secondly for the diploids, tetraploids, and chimeras in each group as determined by the last heart leaf counted. In Figures7B, 8B and 9B the same procedure is followed but the data is plotted as the percent tetraploid cells at the different growth stages.

The average chromosome number at a given growth stage is higher as the number of growth stages analysed decreases (i.e. at the 12th heart leaf the average chromosome number of plants sampled at six stages is 22.4, 26.6 for plants sampled 5 times and 38.2 for plants sampled four

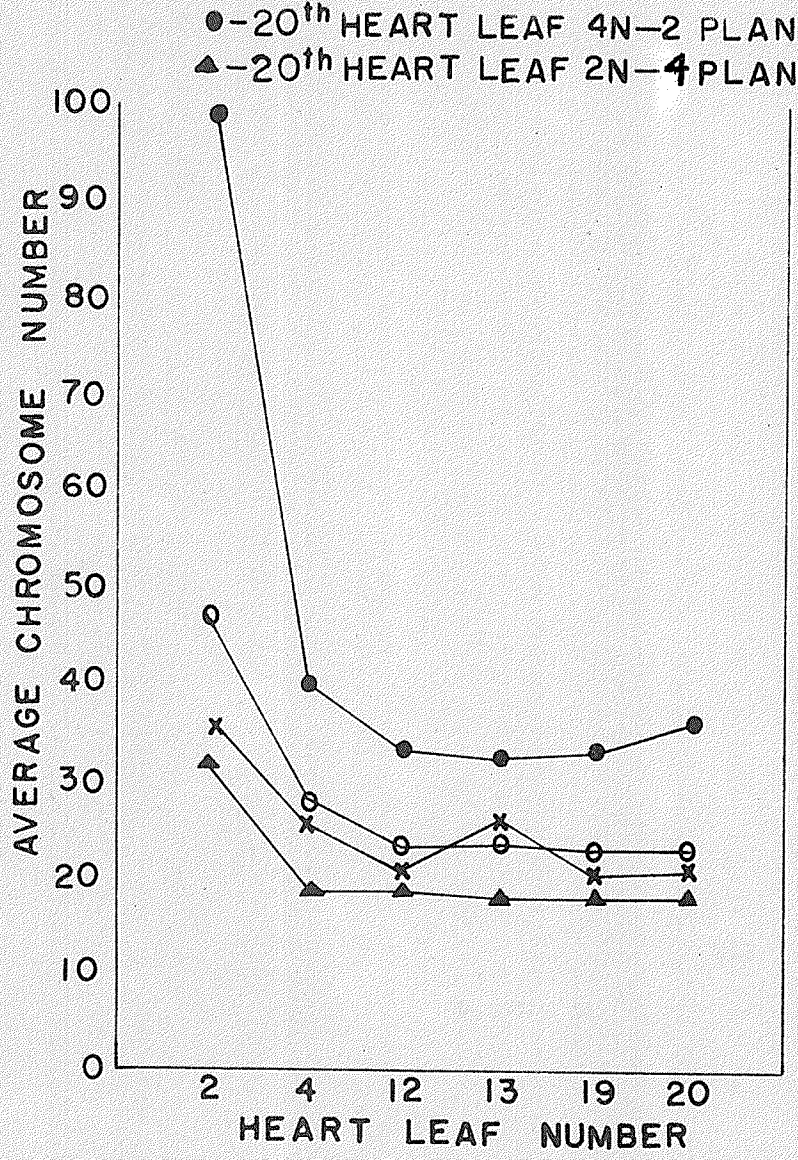

Fig. 7A. - The average chromosome number in heart leaves of 10 Co plants sampled at six growth stages.

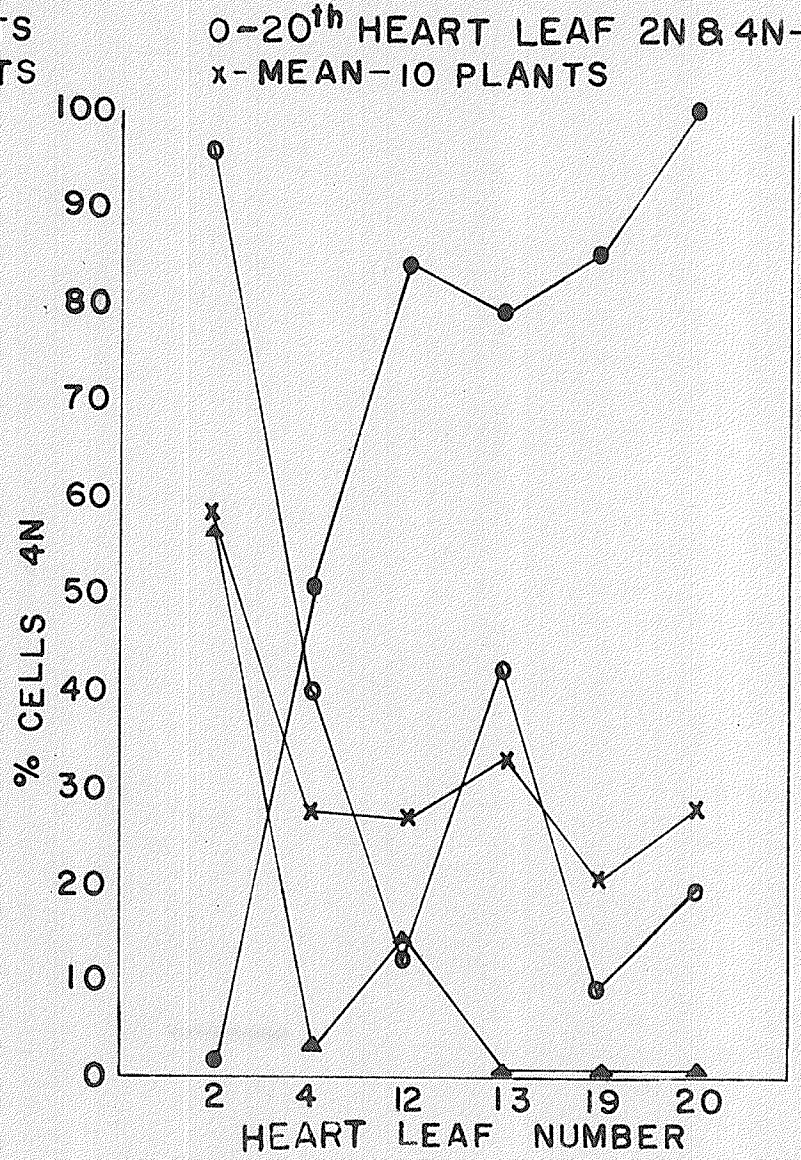

Fig. 7B. - The percent cells 4N in heart leaves of 10 Co plants sampled at six growth stages.

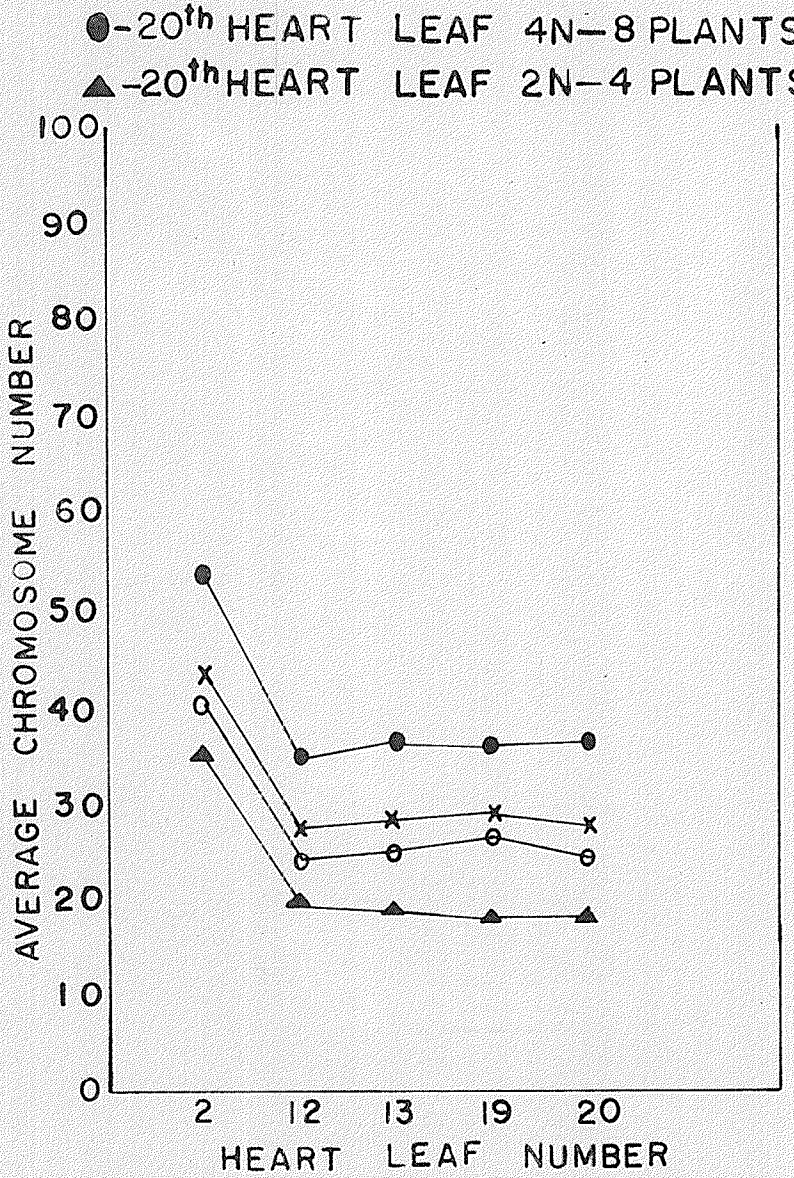

Fig. 8A. - The average chromosome number in heart leaves of 22 Co plants sampled at five growth stages.

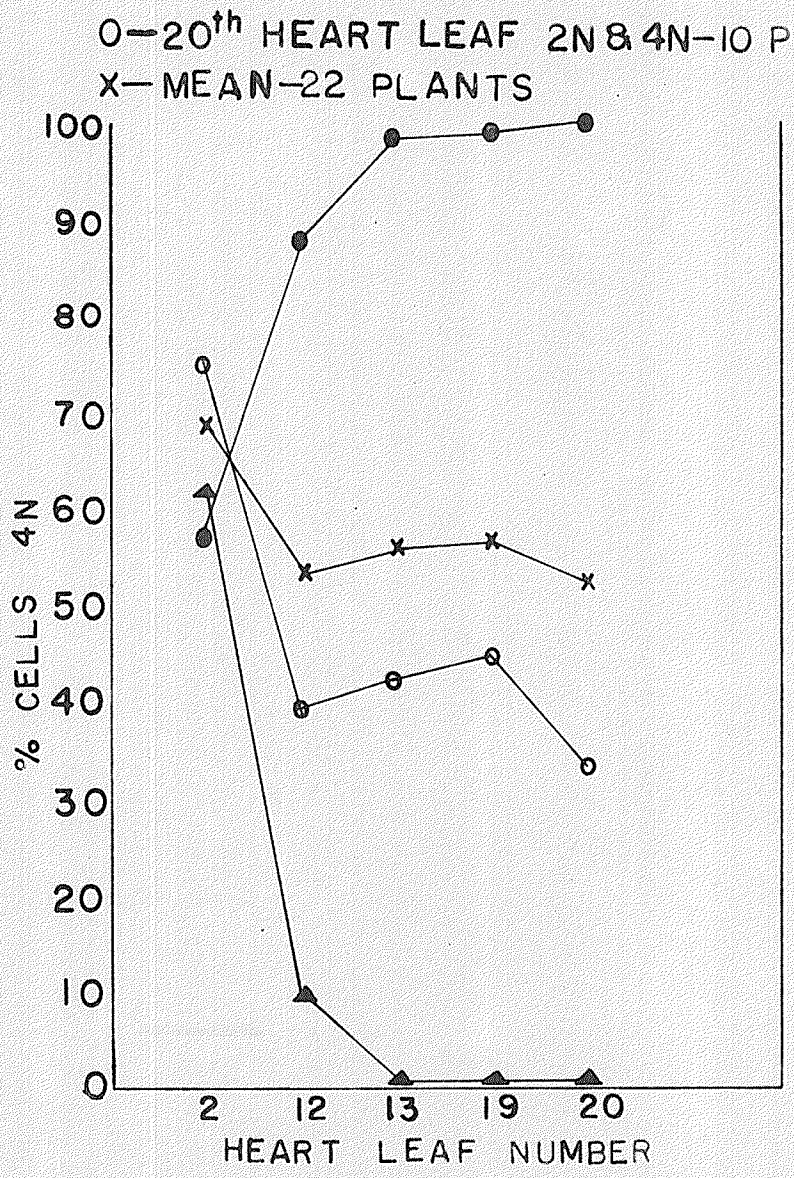

Fig. 8B. - The percent cells 4N in heart leaves of 22 Co plants sampled at five growth stages.

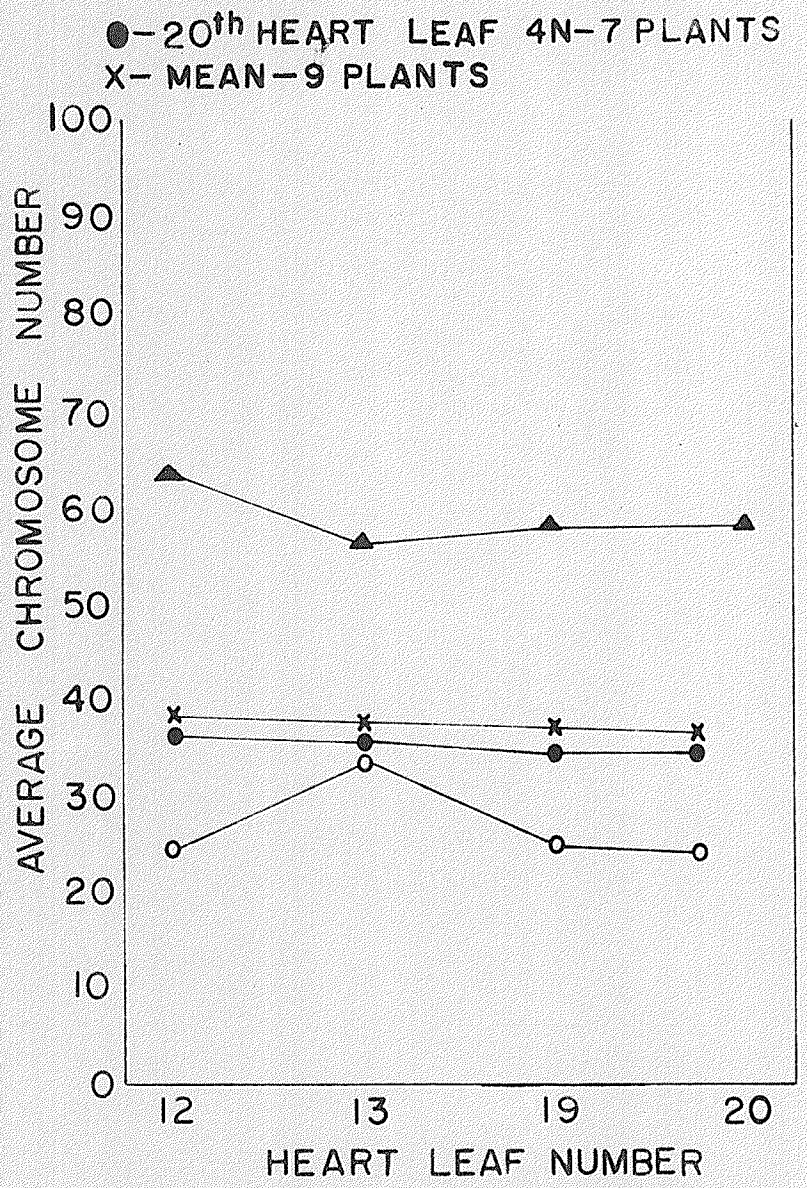

Fig. 9A. – The average chromosome number in heart leaves of nine Co plants sampled at four growth stages.

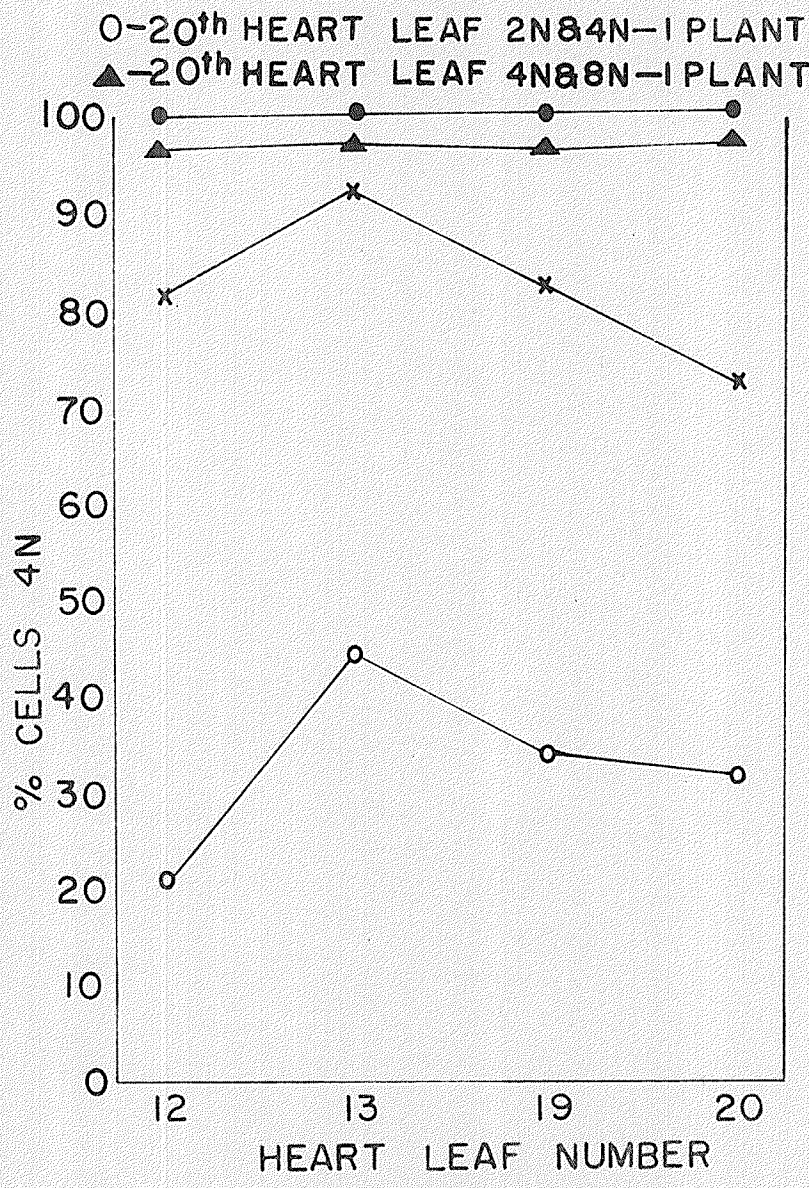

Fig. 9B. – The percent cells 4N in heart leaves of nine Co plants sampled at four growth stages.

times). This is likely due to the fact that the majority of the plants that were analyzable in the early growth stages were relatively less affected by colchicine and thus had a lower chromosome number.

These graphs indicate that the heart leaves have a high average chromosome number in early growth which decreases rapidly and reaches a relatively stable level by the time the 12th heart leaf is sampled. The high chromosome number in the early heart leaves is due to the occurrence of 8N and 16N cells. The frequency of such cells declines rapidly and cells greater than 4N are seldom observed beyond the 4th heart leaf. Table 1 gives the eight groups of chromosome numbers found in the first heart leaves sampled and the number of plants found in each group. Of the 26 plants with heart leaf cells having 72 or 144 chromosomes at the early stages only one plant had heart leaf cells with more than 36 chromosomes at the 12th leaf stage. This plant was a 4N - 8N chimera. The mitotic configurations of a typical 4N - 8N chimera is shown in Plate II. Figures 11 and 12 show mitotic metaphases of 135 and 72 chromosomes respectively, which presumably have arisen through successive C - mitoses. Plate III. Figure 13, shows a mitotic metaphase of 9 chromosomes found in one Co plant at the 20th heart leaf stage. This plant was a chimera of 18, 36 and 9 (Table IV, No. 209) and later produced a 4N inflorescence. The chromosome number of 9 could have arisen by somatic reduction but more likely arose by the formation of a multipolar restitution nucleus (19).

TABLE I

CHROMOSOME NUMBERS FOUND IN EARLIEST HEART LEAF SAMPLED

Chromosome Numbers Found in First Heart Leaf	Number of Plants
18	22
36	5
36 and 18	12
72 and 36	8
36 and 72 and 18	10
144 and 72	1
144 and 36 and 72	6
144	1

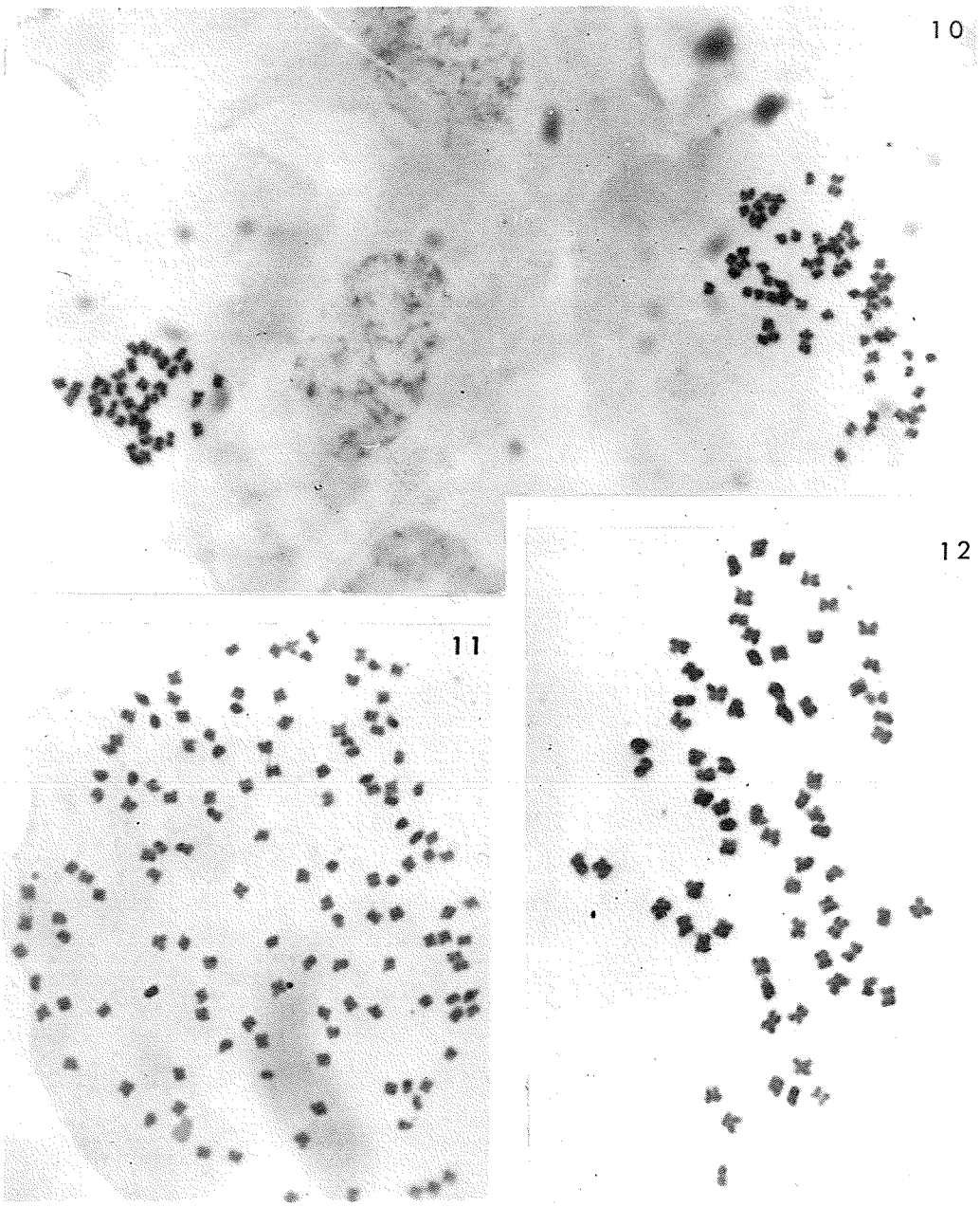

PLATE II - The mitotic metaphases of some heart leaves encountered in the cytological analysis.

Fig. 10 - chimera of 36 and 72 univalents

Fig. 11 - 135 univalents

Fig. 12 - 72 univalents

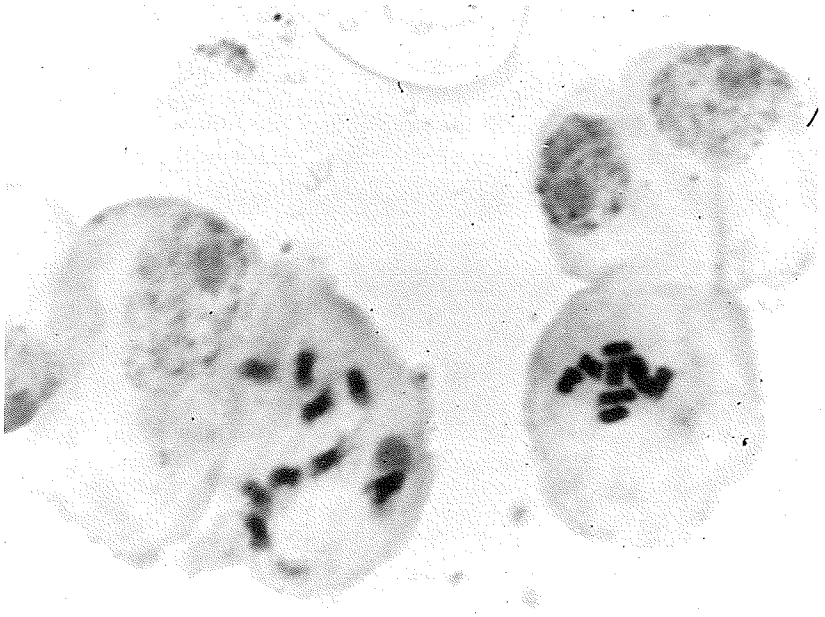

PLATE III -

Fig. 13. - The somatic metaphase of cells with 9 chromosomes found in a chimera leaf of a Co sugar beet.

The possibility of predicting the "chromosome balance" at later stages of plant growth from the "chromosome balance" of an early stage, makes it of interest to compare the relative frequencies of 2N and 4N cells at different growth stages.

The data for the 32 plants in Figure 7A and 8A is regrouped and presented in Table II for three growth stages. The first stage includes data from all the heart leaves sampled prior to the 12th heart leaf. Stages two and three have the combined data from the 12th and 13th, and 19th and 20th heart leaves, respectively. None of these plants had a chromosome number greater than 4N after the 4th heart leaf stage.

Six of the nine plants that were 4N in the 3rd growth stage were completely 4N or greater, and three of them (No. 179, 211 and 184) were chimeras with some 2N cells, at stage one. At stage two all except No. 14 and No. 179 were totally 4N and these two were 75 percent and 95 percent 4N respectively. All of these nine plants analyzed produced 4N inflorescences (Table III). Six of the 17 plants that ended up chimeras showed an increase in percent 4N cells from stage two to stage three and 11 plants showed a decrease. In general, the percent 4N cells decreased and the percent 2N cells increased from stage one to stage two, but the opposite also occurred less often. Two of these plants (No. 4 and 55) produced 4N inflorescences (Table III and IV). All six plants whose last heart leaf was 2N showed a decrease in percent 4N cells with increasing growth stage and all produced 2N inflorescences (of plants analyzed).

TABLE II

THE FREQUENCY OF 2N, 4N AND GREATER THAN 4N CELLS IN HEART LEAVES AT THREE GROWTH STAGES

Plant Number	Stage 1 Heart Leaf 2, 3 and 4			Stage 2 Heart Leaf 12 and 13	Stage 3 Heart Leaf 19 and 20
	% Cells > 4N	% Cells 2N	% Cells 4N	% Cells 4N	% Cells 4N
184	49	2	49	100	100
13	100	0	0	100	100
14	90	0	10	75	100
34	20	0	80	100	100
49	53	0	47	100	100
110	0	0	100	100	100
179	0	32	68	95	100
211	14	12	74	100	100
213	16	0	84	100	100
5	0	24	76	30	95
55	65	33	2	61	85
6	87	0	13	96	66
4	50	5	45	28	53
36	0	0	100	85	44
11	0	26	74	4	32
199	0	40	60	23	22
116	6	0	94	25	22
206	0	46	54	71	20
172	0	0	100	46	20
127	0	5	95	8	15
65	0	31	69	0	14
25	0	2	98	66	13
20	10	0	90	20	11
28	0	63	37	20	8
3	6	66	28	26	6
113	32	0	68	21	6
16	0	0	100	6	0
175	7	23	70	0	0
26	44	19	37	0	0
178	2	68	30	0	0
22	0	88	12	0	0
159	0	89	11	0	0

TABLE III

DATA FROM THE ANALYSIS OF THE FLORAL HEART LEAVES, POLLEN MOTHER CELLS, ROOT TIPS, POLLEN DIAMETER AND CHLOROPLAST NUMBER FROM 34 PLANTS FOUND TO BE TOTALLY 4N IN THE LAST HEART LEAF COUNTED

Plant Number	Last Heart Leaf Number	Frequencies of 4N:2N Cells: Floral Heart Leaves	Pollen Mother Cells	Root Tips	Mean Pollen Diameter in μ	Mean Chloroplast Number per 2 Guard Cells[2]
7	10	50:0	50:0	0:25	26.64	-
13	20	50:0	50:0	0:25	26.02[1]	-
17	20	50:0	50:0	0:25	25.46[1]	-
29	20	50:0	50:0	N.C.	25.67	-
30	20	50:0	50:0	0:25	24.33	-
31	13	50:0	50:0	0:25	27.00[1]	-
34	20	50:0	50:0	0:25	27.28[1]	-
46	19	50:0	50:0	0:25	27.69[1]	-
49	20	50:0	50:0	0:25	27.36[1]	-
55	20	50:0	50:0	0:25	28.98	30.25
59	20	50:0	N.C.	0:25	25.50	-
102	20	50:0	50:0	N.C.	25.94	29.90
110	20	50:0	50:0	N.C.	28.14[1]	29.80
179	20	50:0	50:0	0:25	26.70[1]	20.30
182	13	50:0	50:0	0:25	26.94	24.65
184	20	50:0	50:0	0:25	24.74[1]	31.10
189	20	50:0	50:0	0:25	26.96[1]	-
207	20	50:0	50:0	0:25	26.23[1]	19.80
210	20	50:0	50:0	0:25	22.84[1]	-
211	20	50:0	50:0	0:25	27.84[1]	19.30
212	20	50:0	50:0	0:25	27.08[1]	29.00
214	16	50:0	50:0	0:25	24.72	-
228	11	50:0	50:0	N.C.	27.05[1]	-
237	13	50:0	50:0	N.C.	26.46[1]	-
242	14	50:0	50:0	N.C.	26.27[1]	22.80
108	20	10:40	0:50[4]	0:25	-	-
75	20	50:0	—[4]	0:25	-	-
111	20	50:0	—[4]	N.C.	-	-
213	19	50:0	—[4]	0:25	-	-
255	20	50:0[3]	—[4]	N.C.	-	-
2	20	—[3]	-	N.C.	-	-
14	20	—[3]	-	N.C.	-	-
135	16	—[3]	-	N.C.	-	-
57	20	50:0	—[5]	N.C.	-	-

[1] Mean of 30 measurements

[2] Mean of 20 stomata

[3] Did not bolt

[4] Late bolter

[5] Mechanical damage

N.C. - not countable

TABLE IV

DATA FROM THE ANALYSIS OF THE LAST HEART LEAVES, FLORAL HEART LEAVES, POLLEN MOTHER CELLS, ROOT TIPS, POLLEN DIAMETER AND CHLOROPLAST NUMBER FROM 24 PLANTS WHOSE LAST HEART LEAF COUNTED WAS A CHIMERA

Plant Number	Last Heart Leaf Number	Frequencies of 4N:2N Cells: Last Heart Leaf	Floral Heart Leaves	Pollen Mother Cells	Root Tips	Mean Pollen Diameter in μ	Mean Chloroplast Number per 2 Guard Cells[2]
187	20	45:5	50:0	50:0	0:25	26.58[1]	17.20
36	20	33:17	11:39	0:50	25:0	20.71[1]	22.85
4	20	25:25	37:13	50:0	0:25	26.68[1]	-
66	20	18:32	10:40	0:50	0:25	21.08[1]	23.45
234	16	18:32	10:40	0:50	0:25	20.98[1]	24.00
15	20	17:33	16:34	0:50	N.C.	20.40	27.90
216	20	17:33	11:39	0:50	0:25	21.15[1]	15.15
65	20	14:36	9:41	0:50	0:25	21.17[1]	25.80
170	20	9:41	8:42	N.C.	25:0	19.66[1]	24.60
172	20	9:41	19:31	0:50	0:25	20.55[1]	20.95
199	20	9:41	10:40	0:50	0:25	20.90[1]	25.00
20	20	8:42	6:44	0:50	0:25	21.45[1]	-
28	20	8:42	0:50	0:50	0:25	20.32	-
196	20	8:42	5:45	0:50	0:25	23.07[1]	-
25	20	7:43	6:44	25:25	0:25	20.40[1]	-
206	20	2:48	7:43	0:50	0:25	21.26	-
139	20	36:14	50:0	___[6]	0:25	-	-
11	20	27:23	7:43	___[6]	N.C.	-	-
116	20	8:42	6:44	___[6]	0:25	-	-
71	20	36:14	___[5]	-	0:25	-	-
239	14	30:20[3]	___[5]	-	N.C.	-	-
86	20	31:19[3]	___[5]	-	0:25	-	-
114	20	20:30[3]	___[5]	-	0:25	-	-
209	20	38:5:7[4]	50:0	50:0	0:25	26.46[1]	-

[1] Mean of 30 measurements
[2] Mean of 20 stomata
[3] Frequency of 8N = 4N
[4] Frequency of 4N:2N:N
[5] Did not bolt
[6] Late bolter
N.C. - not countable

These data indicate a general increase in the number of 2N cells with increasing growth stage. However, 4N cells sometimes increase at the expense of 2N cells while both 4N and 2N cells increase relative to cells with chromosome numbers greater than 4N.

There were some plants (No. 6, 13, 14, 36, 116, 172, 20, 113, 16) which have shown all cells in Stage one to be of a higher ploidy than cells that were subsequently found in Stages two and three. Yet there were no instances where Stage one had all cells of a lower chromosome number than that subsequently found in stages two and three. Thus it does not seem logical to attribute the observation of cells not previously encountered to a sectorial chimera in the corpus. The phenomena is more adequately explained by assuming that the primordia of the heart leaves in Stage one were already formed in the embryo at the time of colchicine treatment and thus received a colchicine treatment totally unrelated to that of the plant apex.

Thus the leaves of Stage one could have a higher chromosome number due to increased susceptibility to the colchicine and the effect of colchicine on the chromosome number of these leaves could be completely different than that on the corpus which later gives rise to the heart leaves of Stages two and three.

Some of these 32 plants, plus the 9 sampled at four growth stages and presented in Graph 9, plus additional plants that were analyzed at less than 4 growth stages received the photo-thermal induction treatment. On the basis of the last heart leaf sampled 34 plants were classified as 4N, 24 as chimeras and 14 of the 2N plants were included for a control.

The data obtained from the floral heart leaves, pollen mother cells, pollen measurements, chloroplast counts (Plate IV. Figure 14) and root tip analysis, of these plants is presented in Tables III, IV and V. The last heart leaf analysis for the chimera group is also included.

The floral heart leaves of 30 of the 31 (4N) plants (Table III) that bolted were completely 4N. Only one plant (No. 108) was a chimera at this stage and it produced a diploid inflorescence. This plant was eliminated from the 4N group before anthesis. The pollen mother cells, of the 24 plants in the 4N group that were analyzed, were all 4N.

The floral heart leaves of 16 of the 20 chimera plants (Table IV) that bolted remained chimeras, three were totally 4N (No. 139, 187, 209) and one was diploid (No. 28). Pollen mother cells of 16 of these plants were analyzed. One inflorescence was chimeral (No. 25), 12 were completely diploid and three were tetraploid (No. 187, 4, 209). The 14 plants in the diploid class remained diploid in the floral heart leaves and PMC's. Only one of the 72 plants analyzed(Table IV, No. 25) was chimera in the inflorescence as it had one 2N and one 4N floret. None of the individual flowers sampled were chimeras as judged by the PMC's.

These data indicate that the floral heart leaf of a plant will have the same chromosome number as its prebolting heart leaf. Also if the floral heart leaf is 2N or 4N then the inflorescence will be 2N or 4N respectively. However, if the floral heart leaf is chimera then the inflorescence will likely be completely 2N although occasionally it may be 4N or chimera.

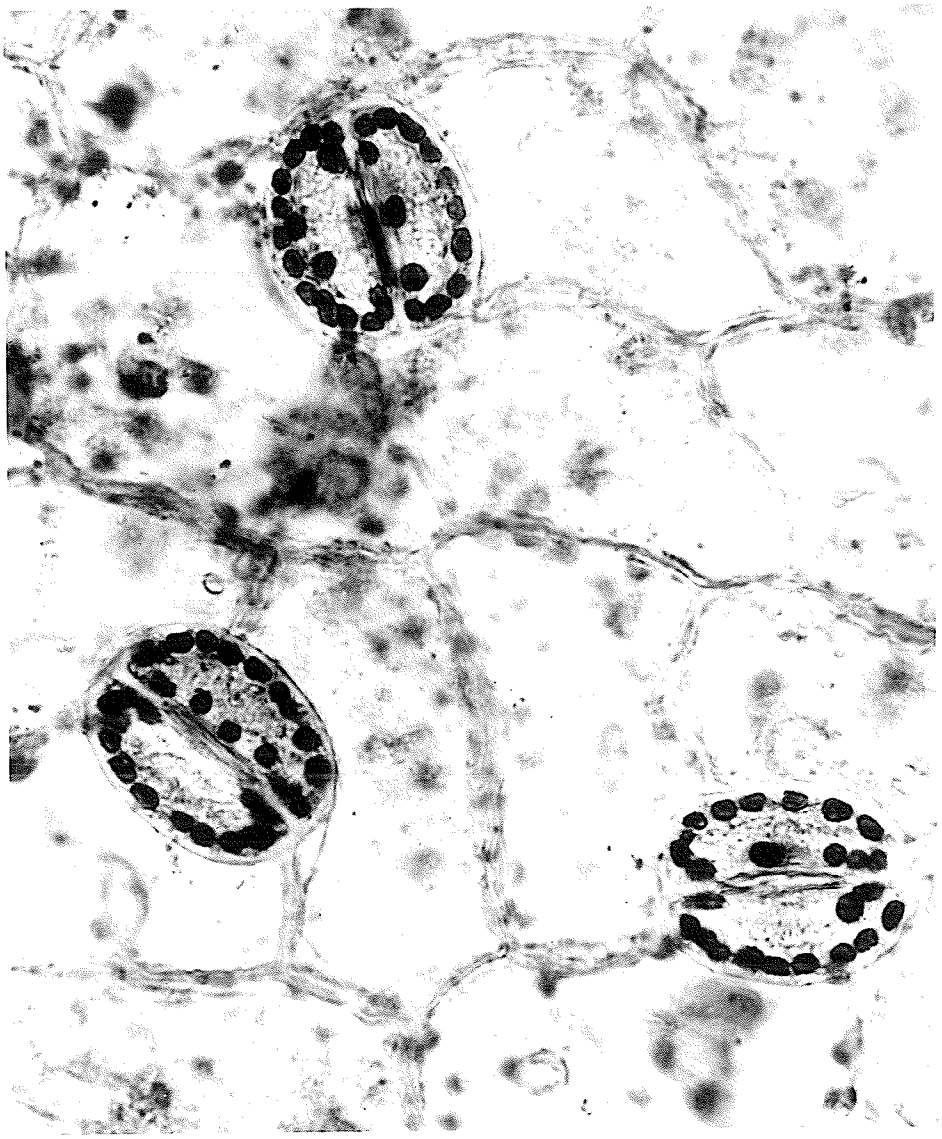

PLATE IV -

Fig. 14 - The lower epidermis of a floral heart leaf showing the guard cells from which chloroplast counts were obtained.

TABLE V

DATA FROM THE ANALYSIS OF THE FLORAL HEART LEAVES, POLLEN MOTHER CELLS, ROOT TIPS, POLLEN DIAMETER AND CHLOROPLAST NUMBER FROM 14 PLANTS FOUND TO BE TOTALLY 2N IN THE LAST HEART LEAF COUNTED

Plant Number	Last Heart Leaf Number	Frequencies of 4N:2N Cells: Floral Heart Leaves	Pollen Mother Cells	Root Tips	Mean Pollen Diameter in μ	Mean Chloroplast Number per 2 Guard Cells[1]
16	20	0:50	0:50	0:25	21.85	17.80
8	20	0:50	0:50	0:25	20.72	16.05
26	20	0:50	0:50	0:25	21.56	17.90
127	20	0:50	0:50	0:25	20.81	15.50
181	20	0:50	0:50	0:25	20.46	17.05
186	20	0:50	0:50	0:25	20.44	17.20
200	20	0:50	0:50	0:25	20.56	16.05
202	20	0:50	0:50	0:25	20.45	15.40
204	20	0:50	0:50	0:25	21.42	-
205	20	0:50	0:50	0:25	21.24	-
220	20	0:50	0:50	0:25	20.54	17.95
178	20	0:50	N.C.	N.C.	20.16	16.95
96	20	——[2]	-	-	-	-
97	20	——[2]	-	0:25	-	-

[1] Mean of 20 stomata

[2] Did not bolt

N.C. - not countable